



ORIGINAL ARTICLE

Gestational age-dependent gene expression profiling of ATP-binding cassette transporters in the healthy human placenta

Guinever E. Imperio^{1,2,3} | Mohsen Javam¹ | Phetcharawan Lye^{1,3} |
 Andrea Constantino¹ | Caroline E. Dunk³  | Fernando M. Reis⁴ |
 Stephen J. Lye^{1,3,5,6} | William Gibb⁷ | Stephen G. Matthews^{1,3,5,6} |
 Tania Maria Ortiga-Carvalho² | Enrrico Bloise^{1,2,8} 

¹Department of Physiology, University of Toronto, Toronto, Ontario, Canada

²Laboratory of Translational Endocrinology, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

³Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

⁴Division of Human Reproduction, Department of Obstetrics and Gynecology, Federal University of Minas Gerais, Belo Horizonte, Brazil

⁵Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada

⁶Department of Medicine, University of Toronto, Toronto, Ontario, Canada

⁷Department of Obstetrics & Gynecology and Department of Cellular & Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada

⁸Department of Morphology, Federal University of Minas Gerais, Belo Horizonte, Brazil

Correspondence

Enrrico Bloise, Department of Morphology N3-292, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
 Email: ebloise@icb.ufmg.br

Funding information

This work was supported by the Bill & Melinda Gates Foundation (OPP1107597) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 401605/2013-2); Canadian Institutes for Health

Abstract

The ATP-binding cassette (ABC) transporters control placental transfer of several nutrients, steroids, immunological factors, chemicals, and drugs at the maternal-fetal interface. We and others have demonstrated a gestational age-dependent expression pattern of two ABC transporters, P-glycoprotein and breast cancer resistance protein throughout pregnancy. However, no reports have comprehensively elucidated the expression pattern of all 50 ABC proteins, comparing first trimester and term human placentae. We hypothesized that placental ABC transporters are expressed in a gestational-age dependent manner in normal human pregnancy. Using the TaqMan[®] Human ABC Transporter Array, we assessed the mRNA expression of all 50 ABC transporters in first (first trimester, n = 8) and third trimester (term, n = 12) human placentae and validated the resulting expression of selected ABC transporters using qPCR, Western blot and immunohistochemistry. A distinct gene expression profile of 30 ABC transporters was observed comparing first trimester vs. term placentae. Using individual qPCR in selected genes, we validated the increased expression of *ABCA1* ($P < 0.01$), *ABCA6* ($P < 0.001$), *ABCA9* ($P < 0.001$) and *ABCC3* ($P < 0.001$), as well as the decreased expression of *ABCB11* ($P < 0.001$) and *ABCG4* ($P < 0.01$) with advancing gestation. One important lipid transporter, *ABCA6*, was selected to correlate protein abundance and characterize tissue localization. *ABCA6* exhibited increased protein expression towards term and was predominantly localized to syncytiotrophoblast cells. In conclusion, expression patterns of placental ABC transporters change as a function of gestational age. These changes are likely fundamental to a healthy pregnancy given the critical role that these transporters play in the regulation of steroidogenesis, immunological responses, and placental barrier function and integrity.

Stephen G. Matthews, Tania Maria Ortiga-Carvalho and Enrrico Bloise contributed equally.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

Research (CIHR; FDN-148368); CNPq (304667/2016-1, 422441/2016-3, 303734/2012-4, 422410/2016-0); Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES); and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, CNE 2015/E26/203.190/2015).

KEYWORDS

ABC transporters, ABCA6, gestational-age, lipid transporter, placenta

1 | INTRODUCTION

The ATP-binding cassette (ABC) superfamily comprise 50 proteins classified into seven sub-families ranging from ABCA through ABCG. Although some of the superfamily members (ABCE and ABCF sub-families) act as translation factors,^{1,2} the majority of the ABC superfamily are transporters, involved in the efflux transport activity of specific substrates across biological barriers.³⁻⁵ In the placenta, ABC transporters regulate fetal accumulation of numerous physiological compounds, chemicals, and drugs that may be present in the maternal circulation.⁶ Endogenous substrates of the ABC transporters include steroid hormones (glucocorticoids, mineralocorticoids, estrogens, androgens, and progestogens), nutrients (lipids, cholesterol, and folate), metabolic products (oxysterols, bilirubin- and bile salts-conjugated compounds) and immunological factors (cytokines and chemokines).^{5,7,8} Exogenous substrates include different environmental chemicals (bisphenol A, ivermectin, organochlorine, and organophosphorus pesticides) and xenobiotics (antiretrovirals, antidepressants, antibiotics, etc).^{5,9-12} Thus, placental ABC transporters control cellular metabolism, regulate local, and systemic immunological responses and orchestrate the biodistribution of numerous endogenous and exogenous substrates at the maternal-fetal interface.^{5,13,14}

The cellular localization of ABC transporters in the syncytiotrophoblast (eg apical vs. basolateral) is essential to determine the transfer orientation of their substrates in or out of the placental barrier.^{5,14-16} P-glycoprotein (P-gp, encoded by *ABCB1*), breast cancer resistance protein (BCRP, *ABCG2*), and the multidrug resistance-associated proteins (MRPs)-2 and 3 (*ABCC2* and *ABCC3*, respectively) are localized to the apical membrane of syncytiotrophoblasts, indicating their extrusion activity occur from within the syncytium (and thus the fetal compartment) towards the maternal blood-enriched intervillous space of the placenta. Conversely, *ABCG1*, *MRP-1*, 3 and 5 (*ABCC1*, *ABCC3*, and *ABCC5*) are localized to the basolateral membrane, suggesting that extrusion activity occurs from the maternal to the fetal compartment.⁵ In some cases, the localization of ABC transporters appears to change with advancing gestation. This is the case for the *ABCA1* lipid transporter, which has been associated with altered steroidogenesis, placental malformation, reduced pregnancies, and offspring morbidity.¹⁷⁻²¹

Considering that ABC transporters are also involved in the biodistribution of several drugs commonly prescribed during pregnancy (ie antiemetic agents, antibiotics, synthetic glucocorticoids,

anti-inflammatory, antidepressants, antihypertensive, and antiretroviral drugs)^{5,22}; a better understanding of the developmental expression of ABC transporters in the placenta may provide important insight concerning drug bioavailability into the fetal compartment throughout pregnancy. We and others have previously demonstrated a time-dependent expression of P-gp, BCRP, *MRP-1*, 2 and 5 in the human placenta throughout pregnancy,^{14,23-30} however, there is no information about the developmental expression of other ABC transporters, highlighting the importance of a more detailed investigation of the expression pattern of the ABC transporter superfamily throughout pregnancy. We hypothesized that healthy human placentae exhibit a developmentally regulated pattern of ABC transporter mRNA expression. Therefore, we sought to comprehensively investigate the developmental expression of the ABC transporter superfamily (50 genes) comparing first trimester and term human placentae from uncomplicated pregnancies.

2 | MATERIALS AND METHODS

2.1 | Sample selection and study design

Healthy human first trimester (7-9 weeks gestation, n = 8) and term (>37 weeks gestation, n = 12) placental samples (paraffin embedded slides and snap-frozen tissues) were obtained from the Research Centre for Women's and Infants' Health (RCWIH) BioBank, after approval of the University of Toronto's Ethics Committee (Protocol #26573). Healthy first trimester placental tissue specimens were obtained following the D&C (dilation and curettage) procedure. Placental villous tissue was visually identified and dissected from other tissues (eg decidua), by highly experienced RCWIH staff. Similarly, placental villous tissue from term pregnancies was dissected and harvested immediately after birth. Placental core sampling was performed by positioning the maternal surface facing up. Dissection was then undertaken in quadrants in areas 1.5 cm away from: the closest placental edge, the center of the placental disc, the umbilical cord insertion site, from areas of thrombosis, infarcts or other abnormalities. The cuts were made to a core depth that excluded the maternal decidua and the chorionic plate. Thus, only placental villous tissue from healthy first and term placentae were included in the study. Placental snap-frozen tissue was stored at -80°C until the time of processing, and paraffin embedded slides were stored dry at room temperature. Considering the privacy policies regulating the collection of specimens by the biobank, first trimester patients'

clinical data were unavailable. However, term patients' clinical data were accessible and described as follows. Maternal average age was 35 ± 1.2 years and BMI was 22 ± 1.1 kg/m². Term pregnancies were 39.5 ± 0.31 weeks and an average birth weight of 3.435 (± 165) g. All term samples were obtained from Caucasian mothers bearing male neonates, to minimize sample heterogeneity.

2.2 | Total RNA extraction and cDNA synthesis

Extraction of total RNA from placental tissue (~30 mg) was performed using the Universal RNeasy Mini kit (Qiagen, Toronto, ON, Canada), in accordance with the manufacturer's instructions. Total RNA concentration was assessed using Nanophotometer and RNA integrity using Experion™ RNA Analysis kit (Bio-Rad, Mississauga, ON, Canada). Samples were used when RNA purity (260/280 absorbance) ratio was >1.8, and RNA integrity number was >7. Total RNA (1 µg) was converted into cDNA using the SuperScript® kit VILO™ cDNA Synthesis (Invitrogen, Grand Island, NY, USA).

2.3 | ABC transporters low density array and individual qPCR

TaqMan® Human ABC Transporter Array microfluidic cards (TLDA, catalog number #4378700; Applied Biosystems, Foster City, CA, USA) were used to analyse placental ABC transporters' mRNA expression, as described previously.³¹ This array was specifically designed to assay gene expression of the 50 ABC transporters and a further 14 reference genes. Briefly, samples were run in triplicate using a total of 10 TLDA cards. Each TLDA card consisted of eight reservoirs (four per sample). A total of 400 ng of reverse transcribed mRNA was used per sample (100 ng/reservoir). cDNA was combined with the 2X Taqman® Gene Expression Master Mix (final volume 100 µL/reservoir) and loaded into separate reservoirs followed by centrifugation. Cards were then sealed and run individually in an Applied Biosystems ViiA™ 7 qPCR System (technologies to Thermo Fisher Scientific, Mississauga, ON, Canada), using the following cycling conditions: 95°C for 20 seconds, followed by 40 cycles of 95°C for 1 second and 60°C for 20 seconds. The geometric mean of the three most stable reference genes (beta-2-microglobulin [B2M], Tata-box binding protein [TBP] and RNA polymerase II subunit A [POLR2A]) was used to normalize the ABC transporter mRNA levels. Cycle thresholds were assessed performed with Thermo Fisher Cloud online software (Life Technologies), the relative expression of target genes was calculated by the $2^{-\Delta\Delta CT}$ method.³² Heatmaps were obtained using R programming statistical software (Foundation for Statistical Computing, Vienna, Austria). A pre-screening was undertaken, comparing samples derived from births at term by vaginal (n = 8) or cesarean section (n = 4). No differences in the relative expression of all 50 ABC transporters were observed between these two modes of delivery, thus all term placental specimens were combined into the Term group. After analysis of relative expression, the resulting *P*-values were corrected for multiple testing by a false discovery rate (FDR)³³ of 5% using the R programming statistical software.

To validate the relative expression results obtained in the array, individual qPCR of selected ABC genes, ABCA1, ABCA6, ABCA9, ABCB1, ABCB11, ABCG4, and ABCC3, was assessed using the same Taqman® probes present in the TLDA cards (ID: Hs00194045_m1, Hs00365329_m1, Hs00184824_m1, Hs00184491_m1, Hs00358656_m1, Hs00223446_m1 and Hs00329320_m1, respectively). Their relative expression was normalized using the gene POLR2A (Hs00172187_m1). qPCR reactions were using the Taqman® Universal Master Mix II (Applied Biosystems) in triplicates in a CFX96 real-time PCR detection system (Bio-Rad). The cycling conditions were: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Changes in mRNA expression were calculated according to the $2^{-\Delta\Delta CT}$ method.³²

2.4 | Western blot

To investigate protein expression of the selected ABC transporter, total protein was extracted from placental tissue (~50 mg) using approaches described previously.³¹ Briefly, nitrocellulose membranes to which protein had been transferred were incubated overnight at 4°C in the presence of a specific primary antibody for the proteins of interest: Anti-ABCA6 (ab180567; Abcam, Toronto, ON, Canada) in a 1:250 PBS dilution with 5% BSA; and anti-ERK (sc-7383; Santa Cruz Biotechnology, Dallas, TX, USA) as an internal control, diluted 1:3000 in PBS with 5% milk. The membrane was then washed, processed, and analysed as described previously.³¹

2.5 | Immunohistochemistry

Mounted paraffin-embedded tissue sections (0.5 µm thickness) were processed as described previously.³¹ Briefly, antigen retrieval was performed by incubating pre-heated (3 minutes in microwave) sections with target retrieval solution, pH 9 (Dako Agilent Technologies, Mississauga, ON, Canada) (2 × 20 minutes on ice); followed by an incubation in sodium citrate solution (10 mmol/L 2 × 15 minutes on ice). Slides were then incubated with Protein Block (Dako) for 1 hour, followed by an overnight 4°C incubation with the primary antibodies: ABCA6 (Abcam, 1:250) and anti-IgG (Dako, used to replace the primary antibody, as a negative control). Sections were then washed in PBS (3 × 5 minutes) and incubated with anti-mouse IgG secondary antibody (X0931; Dako) for 1 hour at room temperature, followed by incubation with streptavidin-HRP (1 hour; Dako) and visualized using diaminobenzidine (Dako). Slides were counterstained with haematoxylin, dehydrated and cover slipped. Sections were examined using an Olympus BX61 upright, motorized microscope coupled with an Olympus DP72 digital camera (Olympus, Tokyo, Japan) at 20X magnification.

2.6 | Statistical analysis

Prism software (GraphPad Software Inc., San Diego, CA, USA) version 5.0 was used for statistical analysis. Analysis included the Kolmogorov-Smirnov normality test followed by an unpaired Student's-t

test or the non-parametric Mann-Whitney test. A 5% FDR was applied to the array data to correct for multiple comparisons. The data displayed refers to the adjusted *P*-value after FDR correction. Specific statistical analysis and the number of samples used are described in the legend of respective figures. Results are expressed as mean \pm SEM. Differences were considered significant when *P* was <0.05 .

3 | RESULTS

3.1 | Time-dependent gene expression of ABC transporters in the human placenta

We observed a dramatic difference in gene expression between first trimester and term placentae, clearly visible in the heatmap (Figure 1) and detailed in Table 1. Healthy placental development was associated with downregulation of 18, and upregulation of 11 ABC transporters (Table 1). *ABCA13*, *ABCB4*, *ABCB9*, *ABCB11*, *ABCC2*, and *ABCG4* were the most decreased, while *ABCA6*, *ABCA8*, *ABCA9*, *ABCA10*, *ABCC3*, and *ABCG1* are the most increased ABC genes (Table 1). As previously observed,³¹ expression of *ABCG5* and *ABCG8* was below detection limit and, therefore, not included in the analysis. *ABCB5*, *ABCC8*, *ABCC12*, and *ABCC13* transcripts exhibited inconsistent amplification results likely because of very low levels of expression, and were not evaluated. Based on their potential

physiological relevance and mRNA abundance (baseline mRNA expression), seven ABC transporter genes were selected for validation using individual qPCR. We confirmed the same pattern previously observed in the array, i.e., significantly increased expression of *ABCA1* ($P < 0.01$), *ABCA6* ($P < 0.001$), *ABCA9* ($P < 0.001$) and *ABCC3* ($P < 0.001$), no difference in *ABCB1* and significantly decreased expression of *ABCB11* ($P < 0.001$) and *ABCG4* ($P < 0.01$), in first trimester compared to term placentae (Figure 2).

3.2 | ABCA6 placental protein expression increases at term and is localized to the syncytiotrophoblast

We next evaluated whether protein levels of a specific ABC transporter, would follow the same pattern as for gene expression. We selected an as yet uncharacterized ABC transporter, the *ABCA6* lipid transporter for analysis, based on its degree of change (fold change: 2.63, $P < 0.001$) and potential physiological relevance for the placenta. As shown in Figure 3A, *ABCA6* exhibited increased ($P < 0.05$) total protein expression at term, demonstrating correspondence between increased transcription and translation of this transporter with advancing gestation. Immunohistochemical analysis indicated that *ABCA6* protein was highly localized to the cytoplasm of the syncytiotrophoblast. Variable *ABCA6* immunoreactivity in the microvillous membrane of the syncytiotrophoblast was also detected, though at a much lower level compared to the cytoplasmic staining. Additionally, some cells of interstitial villi of both first trimester and term placentae were also positive for *ABCA6* (Figure 3B,C).

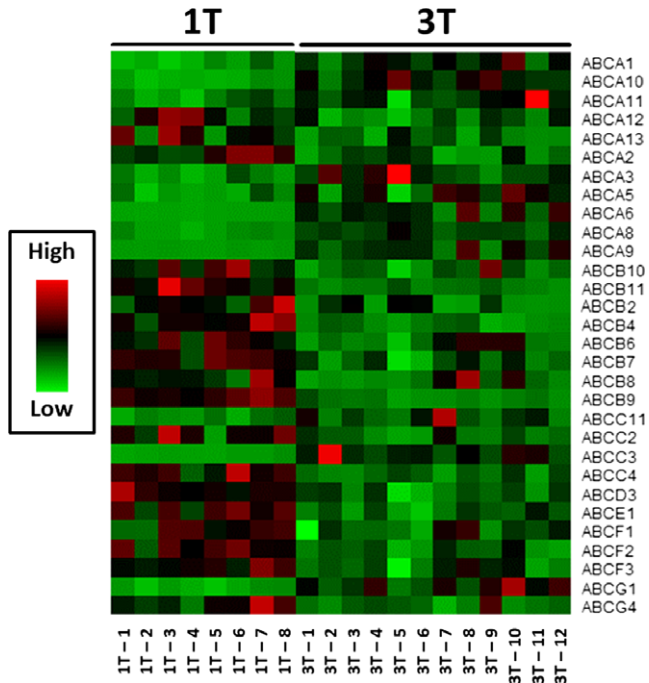


FIGURE 1 Heatmap indicating the time-dependent expression of the 30 ATP-binding cassette (ABC) genes that exhibited developmentally regulated expression in healthy human placenta. Relative quantities (Rq) of the ABC genes obtained from the Human ABC Transporters Taqman[®] Array, comparing first trimester ($n = 8$) and term ($n = 12$) human placentae. Red represents increased expression, green indicates reduced expression

4 | DISCUSSION

In the current study, we show for the first time, a gestational-age specific pattern of expression of 30 ABC transporters in the healthy human placenta, highlighting a potential role for these transporters in regulating placental development, metabolism, and intrauterine disposition of specific substrates throughout pregnancy. Importantly, the lipid transporter *ABCA6* exhibited increased placental mRNA and protein expression towards term and was highly localized to the syncytiotrophoblast in first trimester and term placentae, suggesting that *ABCA6* may have a relevant role supporting placental lipid metabolism and transport, particularly in late pregnancy, when protein levels are at their highest.

Among all the regulated ABC genes, the ABCA subset exhibited the greatest degree of upregulation across pregnancy, particularly for *ABCA6*, *ABCA8*, *ABCA9*, and *ABCA10*. This is perhaps unsurprising, considering that this subfamily of transporters comprises the major lipid transporters responsible for lipid homeostasis,³⁴⁻³⁶ lipid translocation and cell signalling.³⁷⁻⁴⁰ In fact, ABCA transporters are of primary importance for cholesterol transport, which is paramount for normal fetal brain and overall body development.⁴¹⁻⁴⁸ Cholesterol is also the precursor of lipid metabolites such as oxysterols and steroid hormones.⁴⁹⁻⁵² Given that the human placenta lacks the cellular machinery required for cholesterol synthesis,⁵³ and the fact that

TABLE 1 Fold-change in mRNA expression of the ATP-binding cassette (ABC) transporters, obtained from the Human ABC Transporters Taqman[®] Array, comparing term ($n = 12$) to first trimester ($n = 8$) human placentae

Name	Fold-change	Name	Fold-change	Name	Fold-change	Name	Fold-change
ABCA1	2.14***	ABCA12	0.56*	ABCB11	0.16***	ABCD1	1.18
ABCA2	0.62**	ABCA13	0.42*	ABCC1	1.06	ABCD2	1.60
ABCA3	2.38**	ABCB1	0.73	ABCC2	0.48***	ABCD3	0.62***
ABCA4	0.68	ABCB2	0.59**	ABCC3	4.13***	ABCD4	0.92
ABCA5	1.73**	ABCB3	0.92	ABCC4	0.55***	ABCE1	0.64***
ABCA6	2.63***	ABCB4	0.28***	ABCC5	1.12	ABCF1	0.76**
ABCA7	1.25	ABCB6	0.69**	ABCC6	1.29	ABCF2	0.64***
ABCA8	3.74**	ABCB7	0.65***	ABCC7	1.42	ABCF3	0.74***
ABCA9	3.02***	ABCB8	0.83*	ABCC9	0.77	ABCG1	2.73***
ABCA10	4.45***	ABCB9	0.33***	ABCC10	1.07	ABCG2	0.80
ABCA11	1.64*	ABCB10	0.58**	ABCC11	1.83*	ABCG4	0.47**

Fold-change was calculated as the ratio between term and first trimester expression. Fold-change >1 indicates increased expression; <1 indicates decreased expression.

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

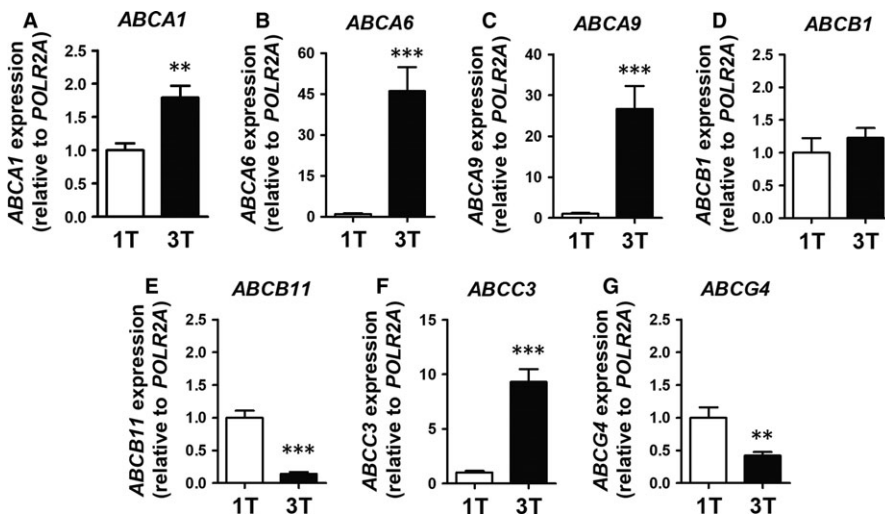


FIGURE 2 Validation of selected ABC genes by qPCR. (A) ABCA1; (B) ABCA6; (C) ABCA9; (D) ABCB1 (E); ABCB11; (F) ABCC3; and (G) ABCG4 mRNA expression, comparing first trimester ($n = 8$) and term ($n = 12$) placentae. Statistical analyses: non-parametric Mann-Whitney test. Data are presented as mean \pm SEM. ** $P < 0.01$ and *** $P < 0.001$

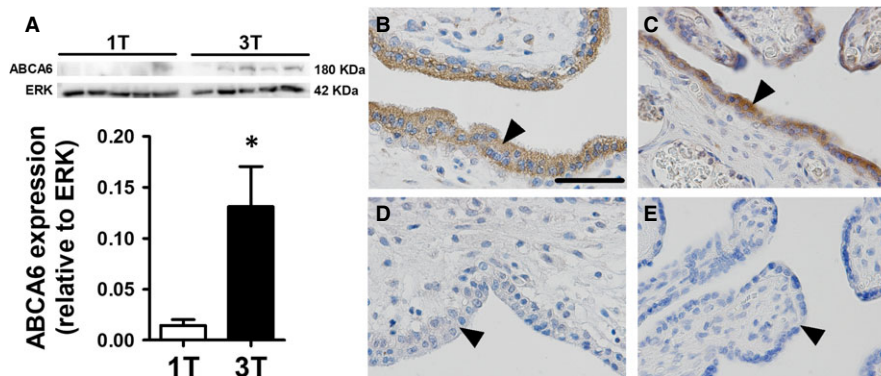


FIGURE 3 Human placental ABCA6 protein expression and immunolocalization towards term. (A) Representative ABCA6 (180 kDa) and ERK (42 kDa) immunoblots, comparing first trimester ($n = 5$; open bar) and term ($n = 5$; solid bar) human placentae, as well as placental ABCA6 expression normalized to ERK (loading control). (B and C) Representative images of ABCA6 localization in first trimester and term placentae, respectively. (D and E) Negative controls for the respective gestational age. Statistical analyses: unpaired t -test. Data are presented as mean \pm SEM * $P < 0.05$, arrows indicate the syncytium, scale bar: 55 μ m

cholesterol can originate from both fetal and maternal compartments,^{54,55} the presence of developmentally-regulated cholesterol transporters in trophoblast cells is likely essential for placental biology. Interestingly, we detected a 2.14-fold increased expression in *ABCA1* mRNA at term compared to first trimester. This finding contrasts with previous published data demonstrating no changes in placental *ABCA1* mRNA levels comparing early vs. late pregnancy placentae.²⁰ These differences may be due to patient selection criteria and or differing sensitivity of the techniques undertaken in both studies.

We selected the *ABCA6* lipid transporter for further protein analysis. *ABCA6* exhibited a marked gestational time-dependent regulation and to the best of our knowledge, has never been previously characterized in the placenta. Placental *ABCA6* protein levels increased with advancing gestation, which paralleled increases in mRNA levels detected by the microfluid array and individual qPCR. Furthermore, *ABCA6* was highly localized to the syncytiotrophoblast cytoplasm regardless of the gestational age. Some sections exhibited variable localization in the microvillous membrane of the syncytiotrophoblast and in interstitial villi cells in both first trimester and term placentae. Considering that *ABCA6* is expressed at Golgi complexes,⁴⁰ our results suggest that *ABCA6* may contribute to lipid homeostasis in the syncytiotrophoblast, probably by transferring cholesterol and oxysterols across the Golgi complex membrane. Furthermore, the variable presence of *ABCA6* in the microvillous membrane of the syncytiotrophoblast suggests that it may also contribute to lipid transport in the placental barrier across pregnancy. However, these hypotheses require further investigation.

Levels of mRNA expression of ABCG genes involved in lipid transport and homeostasis were also developmentally regulated. Of importance, *ABCG1* was highly upregulated in term placentae. *ABCG1* has been demonstrated to synergize with *ABCA1*—which also exhibited higher gene transcript levels in term placentae, to efflux cholesterol and generate HDL particles.⁵⁶

In contrast to the ABCA subset of transporters, the ABCB transporters exhibited the greatest decreases of mRNA throughout pregnancy. Nine out of 11 ABCB transporters were decreased at term, in particular, *ABCB2*, *ABCB4*, and *ABCB9* mRNA. The ABCB subset comprise transporters known to elicit multidrug resistance to neoplastic cells.⁵⁷ In the placenta, ABCB transporters confer embryo/fetal protection against xenobiotics and environmental toxins that may present in the maternal circulation.^{5,14} The best well-characterized placental ABCB transporters are *ABCB1*, which encodes P-gp and *ABCB4*, the MRP-3 encoding gene.⁵ Previously, we have demonstrated a distinct pattern of time-dependent expression of P-gp/*ABCB1* in the human placenta. P-gp is highly expressed in the human placenta and is primarily localized to the apical membrane of the syncytiotrophoblast. P-gp functions as a major efflux transporter that protects the fetus from accumulation of several obstetric-relevant drugs and environmental toxins⁵ and is an important transporter that modulates extravillous trophoblast invasion in early pregnancy.⁵⁸ In our previous studies, syncytiotrophoblast P-gp staining and placental *ABCB1* expression decreased towards term, suggesting a higher

protection of the fetus from exposure to P-gp substrates in the first trimester, a period in which the developing conceptus is most vulnerable to teratogenicity.^{23,59} Our present findings did not recapitulate the previous observed reduced placental *ABCB1* expression towards term. This result from several factors, including patient selection criteria, different specificity of qPCR techniques undertaken, different reference genes used (*POLR2a*, *TBP* and *B2M* vs. *YWHAZ*, *HPRT* and *SDHA*), the evaluation of distinct *ABCB1* transcripts: according to Ensembl Genome Database Project (<http://www.ensembl.org/>), the human *ABCB1* gene has 11 transcripts (splice variants), with four of them being protein coding, which may have led to the evaluation of different *ABCB1* transcripts in these studies. Nevertheless, reduced expression of nine ABCB transporters' genes supports the notion that placental-mediated fetal protection to certain substrates declines in late gestation. In parallel with these decreases in the placenta, there is a dramatic increase in P-gp in the developing fetal blood-brain barrier which confers protection of the brain during the fetal to neonatal transition.^{5,60,61}

ABCB4 (*MDR3*), along with the other downregulated ABC transporters including the bile salt export pump *ABCB11* (BSEP), *ABCC2* (MRP2) and *ABCC4* (MRP4), are components of the hepatobiliary-like excretory system.⁶²⁻⁶⁴ These transporters provide an important route of elimination of toxic compounds produced by fetal metabolism, such as bile acids,^{63,65} that otherwise due to the immaturity of the fetal liver would accumulate and harm the fetus.⁶⁶ Similarly, placental expression of the peroxisomal transporter *ABCD3*, which is involved in fatty acids oxidation and bile acids synthesis⁶⁷ was decreased at term compared to first trimester. Our current results support a previous study,⁶² that suggested placental excretory activity is gradually replaced by fetal hepatic performance with the development and maturation of the fetal liver. This highlights the potential importance of ABC transporters for the clearance control of bile acids and conjugates of bilirubin, bile salts and xenobiotics,^{5,68-73} particularly in earlier stages of pregnancy.

Gene expression of the intracellular ABC proteins, *ABCE1* and *ABCF1-3*, was also decreased in term placentae. While their placental function are not known, our data provide evidence these ABC genes may developmentally regulate intracellular processes in trophoblast cells, which may include ribosomal cycling and protein synthesis⁷⁴ and inflammatory responses to cytosolic DNA (DNA sensing)⁷⁵ and chorioamnionitis.³¹

ABCG2, which encodes the multidrug resistance transporter BCRP, did not show changes in gene expression throughout pregnancy. Like P-gp, BCRP is highly expressed in the apical membrane of the syncytiotrophoblast barrier to confer fetal protection against harmful substances present in the maternal blood; but with an additional role, to regulate cytotrophoblast fusion into syncytiotrophoblasts.⁷⁶ Stable levels of *ABCG2* across pregnancy are consistent with our previous studies.^{23,27} BCRP staining however, is increased in the syncytiotrophoblast towards the end of pregnancy, indicating an increment of fetal protection against BCRP substrates as gestation proceeds.^{23,27}

In conclusion, placental development is associated with a very specific expression pattern of 30 ABC transporter genes. We have also demonstrated a gestational age-dependent pattern of ABCA6 mRNA and protein expression and its abundant localization to the syncytiotrophoblast. Our findings suggest that ABCA6 is likely to exert yet unexplored gestational-age dependent actions in placental lipid homeostasis and transport. Our data also highlight the need for further studies exploring the role of other yet uncharacterized developmentally-regulated ABC transporters in the placenta, which likely exert important actions throughout pregnancy. Considering their crucial role in regulating steroidogenesis, placental nutrient transfer, barrier efficiency and integrity, as well as diverse intracellular processes, the ABC transporters in the placenta likely play critical roles in normal, and pathological pregnancies. This represents a critical area for future research.

AUTHOR'S ROLES

Conception and design: GEI, WG, TMOC, SGM, EB. Acquisition of data, analysis and interpretation of data: GEI, MJ, PL, AC, CD, EB. Drafting the article or revising it critically for important intellectual content: GEI, FMR, WG, SJL, TMOC, SGM, EB. Final approval of the version to be published: GEI, MJ, PL, AC, CD, FMR, WG, SJL, TMOC, SGM, EB.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

The authors thank the donors, RCWIH BioBank, the Lunenfeld-Tanenbaum Research Institute, and the Mount Sinai Hospital/UHN Department of Obstetrics and Gynaecology for the human specimens used in this study (<http://biobank.lunenfeld.ca>). We are also very grateful for Alisa Kostaki basic support in this research.

ORCID

Caroline E. Dunk  <http://orcid.org/0000-0002-4146-099X>

Enrrico Bloise  <http://orcid.org/0000-0003-0275-8686>

REFERENCES

- Kerr ID. Sequence analysis of twin ATP binding cassette proteins involved in translational control, antibiotic resistance, and ribonuclease L inhibition. *Biochem Biophys Res Commun*. 2004;315:166-173.
- Chen ZQ, Dong J, Ishimura A, Daar I, Hinnebusch AG, Dean M. The essential vertebrate ABCE1 protein interacts with eukaryotic initiation factors. *J Biol Chem*. 2006;281:7452-7457.
- Allikmets R, Dean M. Cloning of novel ABC transporter genes. *Methods Enzymol*. 1998;292:116-130.
- Tarling EJ, de Aguiar Vallim TQ, Edwards PA. Role of ABC transporters in lipid transport and human disease. *Trends Endocrinol Metab*. 2013;24:342-350.
- Bloise E, Ortiga-Carvalho TM, Reis FM, Lye SJ, Gibb W, Matthews SG. ATP-binding cassette transporters in reproduction: a new frontier. *Hum Reprod Update*. 2016;22:164-181.
- Mairinger S, Erker T, Muller M, Langer O. PET and SPECT radiotracers to assess function and expression of ABC transporters in vivo. *Curr Drug Metab*. 2011;12:774-792.
- Barnes KM, Dickstein B, Cutler GB, Fojo T, Bates SE. Steroid treatment, accumulation, and antagonism of P-glycoprotein in multidrug-resistant cells. *Biochemistry*. 1996;35:4820-4827.
- Gill S, Chow R, Brown AJ. Sterol regulators of cholesterol homeostasis and beyond: the oxysterol hypothesis revisited and revised. *Prog Lipid Res*. 2008;47:391-404.
- Schinkel AH, Smit JJ, van Tellingen O, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell*. 1994;77:491-502.
- Aurade RM, Jayalakshmi SK, Sreeramulu K. P-glycoprotein ATPase from the resistant pest, *Helicoverpa armigera*: purification, characterization and effect of various insecticides on its transport function. *Biochim Biophys Acta*. 2010;1798:1135-1143.
- Jin H, Audus KL. Effect of bisphenol A on drug efflux in BeWo, a human trophoblast-like cell line. *Placenta*. 2005;26(Suppl A):S96-S103.
- Yoshikawa Y, Hayashi A, Inai M, Matsushita A, Shibata N, Takada K. Permeability characteristics of endocrine-disrupting chemicals using an in vitro cell culture model, Caco-2 cells. *Curr Drug Metab*. 2002;3:551-557.
- van de Ven R, Oerlemans R, van der Heijden JW, et al. ABC drug transporters and immunity: novel therapeutic targets in autoimmunity and cancer. *J Leukoc Biol*. 2009;86:1075-1087.
- Iqbal M, Audette MC, Petropoulos S, Gibb W, Matthews SG. Placental drug transporters and their role in fetal protection. *Placenta*. 2012;33:137-142.
- Petropoulos S, Kalabis GM, Gibb W, Matthews SG. Functional changes of mouse placental multidrug resistance phosphoglycoprotein (ABCB1) with advancing gestation and regulation by progesterone. *Reprod Sci*. 2007;14:321-328.
- Petropoulos S, Gibb W, Matthews SG. Developmental expression of multidrug resistance phosphoglycoprotein (P-gp) in the mouse fetal brain and glucocorticoid regulation. *Brain Res*. 2010;1357:9-18.
- McNeish J, Aiello RJ, Guyot D, et al. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc Natl Acad Sci U S A*. 2000;97:4245-4250.
- Christiansen-Weber TA, Volland JR, Wu Y, et al. Functional loss of ABCA1 in mice causes severe placental malformation, aberrant lipid distribution, and kidney glomerulonephritis as well as high-density lipoprotein cholesterol deficiency. *Am J Pathol*. 2000;157:1017-1029.
- Albrecht C, Nikitina L, Wenger F, Baumann M, Surbek D, Körner M. Localisation of ABCA1 in first trimester and term placental tissues. *Placenta*. 2010;31:741-742.
- Bhattacharjee J, Ietta F, Giacomello E, et al. Expression and localization of ATP binding cassette transporter A1 (ABCA1) in first trimester and term human placenta. *Placenta*. 2010;31:423-430.
- Nikitina L, Wenger F, Baumann M, Surbek D, Körner M, Albrecht C. Expression and localization pattern of ABCA1 in diverse human placental primary cells and tissues. *Placenta*. 2011;32:420-430.
- Marquez B, Van Bambeke F. ABC multidrug transporters: target for modulation of drug pharmacokinetics and drug-drug interactions. *Curr Drug Targets*. 2011;12:600-620.

23. Lye P, Bloise E, Dunk C, et al. Effect of oxygen on multidrug resistance in the first trimester human placenta. *Placenta*. 2013;34:817-823.
24. Mathias AA, Hitti J, Unadkat JD. P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages. *Am J Physiol Regul Integr Comp Physiol*. 2005;289:R963-R969.
25. Gil S, Saura R, Forestier F, Farinotti R. P-glycoprotein expression of the human placenta during pregnancy. *Placenta*. 2005;26:268-270.
26. Mylona P, Hoyland JA, Sibley CP. Sites of mRNA expression of the cystic fibrosis (CF) and multidrug resistance (MDR1) genes in the human placenta of early pregnancy: no evidence for complementary expression. *Placenta*. 1999;20:493-496.
27. Yeboah D, Sun M, Kingdom J, et al. Expression of breast cancer resistance protein (BCRP/ABCG2) in human placenta throughout gestation and at term before and after labor. *Can J Physiol Pharmacol*. 2006;84:1251-1258.
28. Meyer zu Schwabedissen HE, Jedlitschky G, Gratz M, et al. Variable expression of MRP2 (ABCC2) in human placenta: influence of gestational age and cellular differentiation. *Drug Metab Dispos*. 2005;33:896-904.
29. Meyer zu Schwabedissen HE, Grube M, Heydrich B, et al. Expression, localization, and function of MRP5 (ABCC5), a transporter for cyclic nucleotides, in human placenta and cultured human trophoblasts: effects of gestational age and cellular differentiation. *Am J Pathol*. 2005;166:39-48.
30. Pascolo L, Ferneti C, Pirulli D, Crovella S, Amoroso A, Tiribelli C. Effects of maturation on RNA transcription and protein expression of four MRP genes in human placenta and in BeWo cells. *Biochem Biophys Res Commun*. 2003;303:259-265.
31. do Imperio GE, Bloise E, Javam M, et al. Chorioamnionitis induces a specific signature of placental ABC transporters associated with an increase of miR-331-5p in the human preterm placenta. *Cell Physiol Biochem*. 2018;45:591-604.
32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25:402-408.
33. Yoav B, Yosef H. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B*. 1995;57:289-300.
34. Albrecht C, Viturro E. The ABCA subfamily—gene and protein structures, functions and associated hereditary diseases. *Pflugers Arch*. 2007;453:581-589.
35. Wenzel JJ, Kaminski WE, Piehler A, Heimerl S, Langmann T, Schmitz G. ABCA10, a novel cholesterol-regulated ABCA6-like ABC transporter. *Biochem Biophys Res Commun*. 2003;306:1089-1098.
36. Wenzel JJ, Piehler A, Kaminski WE. ABC A-subclass proteins: gatekeepers of cellular phospho- and sphingolipid transport. *Front Biosci*. 2007;12:3177-3193.
37. Albrecht C, McVey JH, Elliott JI, et al. A novel missense mutation in ABCA1 results in altered protein trafficking and reduced phosphatidylserine translocation in a patient with Scott syndrome. *Blood*. 2005;106:542-549.
38. Flieger O, Engling A, Bucala R, Lue H, Nickel W, Bernhagen J. Regulated secretion of macrophage migration inhibitory factor is mediated by a non-classical pathway involving an ABC transporter. *FEBS Lett*. 2003;551:78-86.
39. Birge RB, Boeltz S, Kumar S, et al. Phosphatidylserine is a global immunosuppressive signal in efferocytosis, infectious disease, and cancer. *Cell Death Differ*. 2016;23:962-978.
40. Gai J, Ji M, Shi C, et al. FoxO regulates expression of ABCA6, an intracellular ATP-binding-cassette transporter responsive to cholesterol. *Int J Biochem Cell Biol*. 2013;45:2651-2659.
41. Ming JE, Roessler E, Muenke M. Human developmental disorders and the Sonic hedgehog pathway. *Mol Med Today*. 1998;4:343-349.
42. Schmitz G, Kaminski WE, Orsó E. ABC transporters in cellular lipid trafficking. *Curr Opin Lipidol*. 2000;11:493-501.
43. Schmitz G, Kaminski WE. ABC transporters and cholesterol metabolism. *Front Biosci*. 2001;6:D505-D514.
44. Lindegaard ML, Wassif CA, Vaisman B, et al. Characterization of placental cholesterol transport: ABCA1 is a potential target for in utero therapy of Smith-Lemli-Opitz syndrome. *Hum Mol Genet*. 2008;17:3806-3813.
45. Aye IL, Singh AT, Keelan JA. Transport of lipids by ABC proteins: interactions and implications for cellular toxicity, viability and function. *Chem Biol Interact*. 2009;180:327-339.
46. Aye IL, Waddell BJ, Mark PJ, Keelan JA. Placental ABCA1 and ABCG1 transporters efflux cholesterol and protect trophoblasts from oxysterol induced toxicity. *Biochim Biophys Acta*. 2010;1801:1013-1024.
47. Yvan-Charvet L, Wang N, Tall AR. Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses. *Arterioscler Thromb Vasc Biol*. 2010;30:139-143.
48. Plösch T, van Straten EM, Kuipers F. Cholesterol transport by the placenta: placental liver X receptor activity as a modulator of fetal cholesterol metabolism? *Placenta*. 2007;28:604-610.
49. Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. *Science*. 1996;274:255-259.
50. Chiang C, Litingtung Y, Lee E, et al. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature*. 1996;383:407-413.
51. Huang X, Litingtung Y, Chiang C. Region-specific requirement for cholesterol modification of sonic hedgehog in patterning the telencephalon and spinal cord. *Development*. 2007;134:2095-2105.
52. Schroepfer GJ. Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol Rev*. 2000;80:361-554.
53. Pasqualini JR, Chetrite GS. The formation and transformation of hormones in maternal, placental and fetal compartments: biological implications. *Horm Mol Biol Clin Invest*. 2016;27:11-28.
54. Napoli C, D'Armiento FP, Mancini FP, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest*. 1997;100:2680-2690.
55. Herman GE. Disorders of cholesterol biosynthesis: prototypic metabolic malformation syndromes. *Hum Mol Genet*. 2003;12:75R-88.
56. Kruit JK, Wijesekera N, Westwell-Roper C, et al. Loss of both ABCA1 and ABCG1 results in increased disturbances in islet sterol homeostasis, inflammation, and impaired β -cell function. *Diabetes*. 2012;61:659-664.
57. Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. *Hum Genomics*. 2009;3:281-290.
58. Dunk CE, Pappas JJ, Lye P, et al. P-Glycoprotein (P-gp)/ABCB1 plays a functional role in extravillous trophoblast (EVT) invasion and is decreased in the pre-eclamptic placenta. *J Cell Mol Med*. 2018. <https://doi.org/10.1111/jcmm.13810>
59. Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of the multidrug resistance P-glycoprotein, (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. *Placenta*. 2006;27:602-609.
60. Moisiadis VG, Matthews SG. Glucocorticoids and fetal programming part 2: Mechanisms. *Nat Rev Endocrinol*. 2014;10:403-411.
61. Moisiadis VG, Matthews SG. Glucocorticoids and fetal programming part 1: outcomes. *Nat Rev Endocrinol*. 2014;10:391-402.
62. Patel P, Weerasekera N, Hitchins M, Boyd CA, Johnston DG, Williamson C. Semi quantitative expression analysis of MDR3, FC1, BSEP, OATP-A, OATP-C, OATP-D, OATP-E and NTCP gene transcripts in 1st and 3rd trimester human placenta. *Placenta*. 2003;24:39-44.

63. Marin JJ, Macias RI, Serrano MA. The hepatobiliary-like excretory function of the placenta. A review. *Placenta*. 2003;24:431-438.
64. Macias RI, Marin JJ, Serrano MA. Excretion of biliary compounds during intrauterine life. *World J Gastroenterol*. 2009;15:817-828.
65. Colombo C, Zuliani G, Ronchi M, Breidenstein J, Setchell KD. Biliary bile acid composition of the human fetus in early gestation. *Pediatr Res*. 1987;21:197-200.
66. Serrano MA, Macias RI, Briz O, et al. Expression in human trophoblast and choriocarcinoma cell lines, BeWo, Jeg-3 and JAr of genes involved in the hepatobiliary-like excretory function of the placenta. *Placenta*. 2007;28:107-117.
67. Wanders RJ, Waterham HR. Biochemistry of mammalian peroxisomes revisited. *Annu Rev Biochem*. 2006;75:295-332.
68. Cuperus FJ, Claudel T, Gautherot J, Halilbasic E, Trauner M. The role of canalicular ABC transporters in cholestasis. *Drug Metab Dispos*. 2014;42:546-560.
69. Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev*. 2003;83:633-671.
70. Azzaroli F, Mennone A, Feletti V, et al. Clinical trial: modulation of human placental multidrug resistance proteins in cholestasis of pregnancy by ursodeoxycholic acid. *Aliment Pharmacol Ther*. 2007;26:1139-1146.
71. Marín JJ, Macías RI, Briz O, Pérez MJ, Serrano MA. Molecular bases of the excretion of fetal bile acids and pigments through the fetal liver-placenta-maternal liver pathway. *Ann Hepatol*. 2005;4:70-76.
72. Marin JJ, Macias RI, Briz O, et al. Molecular bases of the fetal liver-placenta-maternal liver excretory pathway for cholephilic compounds. *Liver Int*. 2008;28:435-454.
73. Ferdinandusse S, Jimenez-Sanchez G, Koster J, et al. A novel bile acid biosynthesis defect due to a deficiency of peroxisomal ABCD3. *Hum Mol Genet*. 2014;24:361-370.
74. Schuller AP, Green R. The ABC(E1)s of ribosome recycling and reinitiation. *Mol Cell*. 2017;66:578-580.
75. Lee MN, Roy M, Ong SE, et al. Identification of regulators of the innate immune response to cytosolic DNA and retroviral infection by an integrative approach. *Nat Immunol*. 2013;14:179-185.
76. Lye P, Bloise E, Nadeem L, Gibb W, Lye SJ, Matthews SG. Glucocorticoids modulate multidrug resistance transporters in the first trimester human placenta. *J Cell Mol Med*. 2018;22:3652-3660.

How to cite this article: Imperio GE, Javam M, Lye P, et al. Gestational age-dependent gene expression profiling of ATP-binding cassette transporters in the healthy human placenta. *J Cell Mol Med*. 2019;23:610–618. <https://doi.org/10.1111/jcmm.13966>